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**MECHANISMS OF HAIR FOLLICLE CYCLE REGULATED BY Wnt-10b
IN REX-RABBITS FROM BIRTH TO 2-MONTH-OLD.**

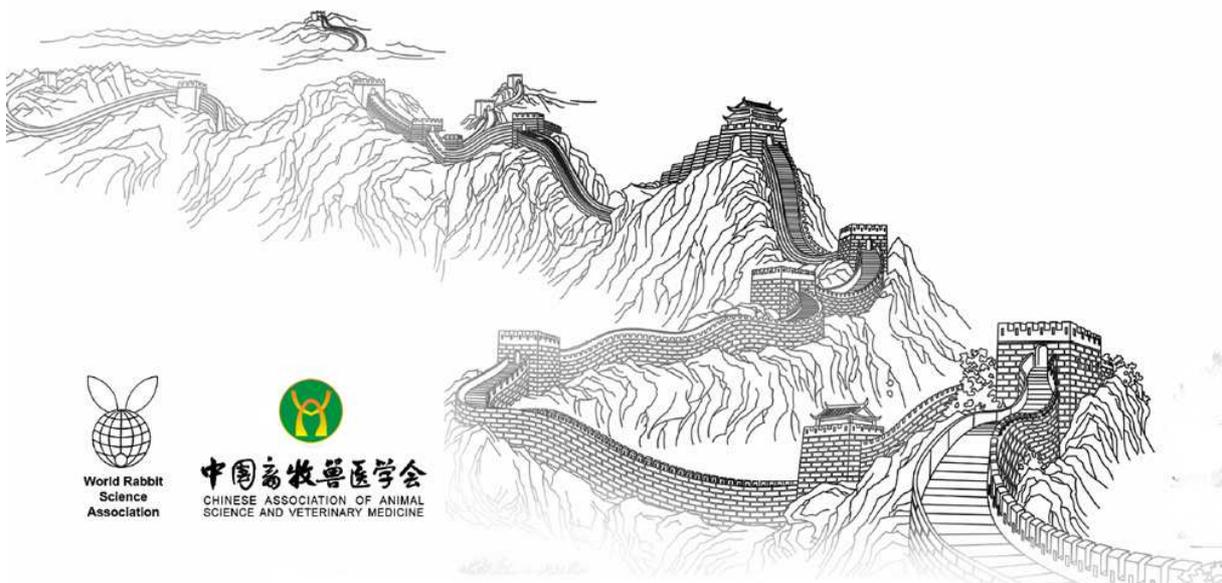
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THE MECHANISMS OF HAIR FOLLICLE CYCLE REGULATED BY WNT10B IN REX-RABBITS FROM BIRTH TO 2-MONTH-OLD

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ABSTRACT

To evaluate the role of Wnt-10b in the skin of Rex-rabbit, we investigated the protein expression of Wnt-10b, β -catenin and Dishevelled (DSH) on Rex-rabbit from birth to 2-month old. Whole skin samples were obtained from the back of rex rabbits at 0, 2, 4, 6 and 8 weeks old. The western blot results showed that, compared with control group (0 week old), the ratio of Wnt-10b protein expression decreased significantly at 2 weeks old and 4 weeks old. Meanwhile, the β -catenin and DSH protein expression also decreased significantly at 2 weeks old and 4 weeks old. Paraffin sections of the skin from each rabbit were immunohistochemically stained to investigate the expression of Wnt-10b protein during the hair follicle cycle of Rex-rabbit from birth to 2-month old. We found that Wnt-10b protein express mainly in hair matrix, IRS and ORS (inner and outer root sheath) at 0, 6 and 8 weeks old. However, it only express in IRS and ORS at 2 and 4 weeks old. Our results suggest that Wnt-10b may play an important role during the hair follicle cycle of the Rex-rabbit through the Wnt/ β -catenin pathway.

Key words: Rex rabbit, Hair follicle, Wnt-10b, β -catenin, DSH, Wnt/ β -catenin

INTRODUCTION

Wnt protein are secreted, lipid-modified glycoproteins that activate cell-surface receptor-mediated signal transduction pathways to regulate a variety of cellular activities including cell fate determination, proliferation, migration, polarity, and gene expression (Alonso, 2003). These proteins are heavily involved in morphogenesis during embryogenesis, as well as in the regeneration of various adult tissues including skin and hair follicles (Peifer, 2000).

Mouse Wnt-10b was initially isolated from lymphoid tissue and suggested to be involved in lymphoid development (Hardiman, 1996; Staal, 2000). And then it was shown to play various roles in a wide range of biological actions, such as adipogenesis (Ross, 2000; Bennett, 2003), bone formation (Bennett, 2005), tumor development (Polakis, 2000; Bienz, 2000; Brennan, 2004), and axis determination (Ishikawa, 2001). Recently, it was found that Wnt-10b is unique and plays an important role in the hair follicle.

However, few studies have been conducted concerning its role in the rabbit. Our study is to reveal the mechanisms of hair follicle cycle regulated by Wnt-10b in Rex-rabbits form birth to 2-month-old.

MATERIALS AND METHODS

Animals and experimental design

All the treatments used to animals in this study approved by Animal Welfare and Healthy Breed Committee of Shandong Province.

During the trials, the rabbits were housed in a closed and ventilated building in which the maximum temperature was 22 °C, the minimum temperature was 15 °C and the relative humidity ranged from 50% to 60%. A cycle of 12 h (from 6:30 to 18:30) of light and 12 h of dark was used during the trial.

The diets were formulated according to the requirements of rabbit does (De Blas and Wiseman, 2010). The feed was pressed using a pellet mill into pellets with diameters of 4 mm.

Rabbit was recorded as 0 week at birth and whole skin samples were obtained from the back of rabbit at 0, 2, 4, 6, 8 weeks old (6 rabbits per stage and equal ration of males: females). 1cm³ skin samples were taken from the mid back and immediately frozen in liquid nitrogen. Subsequently, they were transported to laboratory in low temperature environment and stored at -80°C. Another 1cm³ skin samples were taken from the mid back and then dropped into the 4% paraformaldehyde.

Chemical Analyses

Western blot analysis

Analyses of Wnt-10b, β -catenin and DSH proteins were performed by western blotting. The frozen skin samples were homogenized and then lysed using a strong Radio Immunoprecipitation Assay lysis buffer and Phenylmethanesulfonyl fluoride (Beyotime, Shanghai, China).

Immunohistochemical analysis

The skins were removed, trimmed of fat and connective tissue, buried in 4% paraformaldehyde overnight separately, and the skin sections were fixed in cold ethanol and embedded in paraffin. Sections (5 μ m thickness) were cut onto gelatinised slides, which were then dried and stored at 4 °C.

Statistical Analysis

All the data were analysed with SAS software (SAS version 8e; SAS Institute, Cary, NC, USA). A one-way ANOVA model was used to evaluate the means among various groups. For all mRNA and protein levels analysis, n=6. The data were shown as the mean \pm SEM. $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Total protein was extracted to analyse the protein expression. The western blot results showed that, compared with control group (0 week old), the ratio of Wnt-10b protein expression decreased significantly at 2 weeks old and 4 weeks old ($P < 0.05$) (Fig 1). Meanwhile, the β -catenin and DSH protein expression also decreased significantly at 2 weeks old and 4 weeks old ($P < 0.05$) (Fig 2, Fig 3).

Paraffin sections of the skin from each rabbit were immunohistochemically stained to investigate the expression of Wnt-10b protein during the hair follicle cycle of Rex-rabbit from birth to 2-month old. We found that Wnt-10b protein express mainly in hair matrix, IRS and ORS (inner and outer root sheath) at 0, 6 and 8 weeks old. However, it only express in IRS and ORS at 2 and 4 weeks old.

Hair follicle is one of the important appendages in skin with a complex structure, including epithelial part (matrix and concentric multiple layers structures) and dermal part (dermal papilla and connective tissue sheath). Hair follicles keep on growth and regeneration by periodic hair cycle which consists of three phases: anagen, catagen and telogen. The hair follicle cycle is strictly regulated by various growth factors, hormones, signal molecules and signaling pathways, such as Wnt, Bmp, Shh, of which canonical Wnt/ β -catenin pathway is one of the most important pathways. Up to now, 19 Wnt members have been identified in mice and humans (Moon, 2002).

It has been carried out a comprehensive survey of all currently identified mouse Wnt genes expression in embryonic and postnatal skin and been identified three Wnt gens, Wnt-10a, 10b and 5a, whose expression is specifically upregulated in hair follicles at early morphogenetic stages. Of these, Wnt-10b shows dramatic upregulation at the earliest stage of hair follicle development and is also specifically expressed in the postnatal hair follicles at the onset of a new cycle of hair growth (Reedy, 2001). Furthermore, other reports showed that Wnt-10b promoted the differentiation of primary skin epithelial cells toward hair shaft and inner root sheath of the hair follicle cells in vitro, elongation of the hair shaft in hair follicle cultures, and induction of hair follicle formation in the cultured embryonic skin tissue.

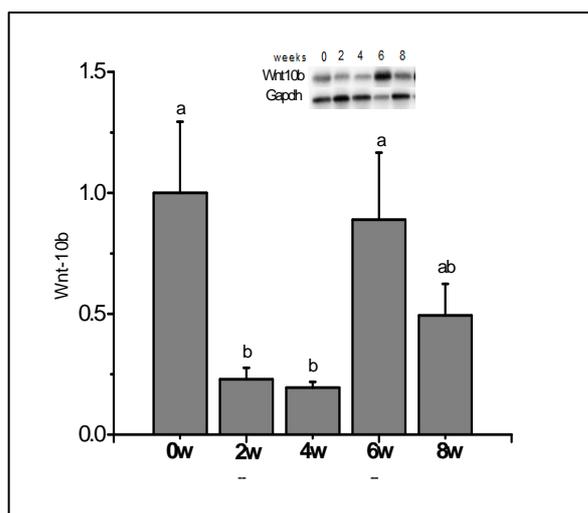


Figure 1: The level of Wnt-10b protein expression

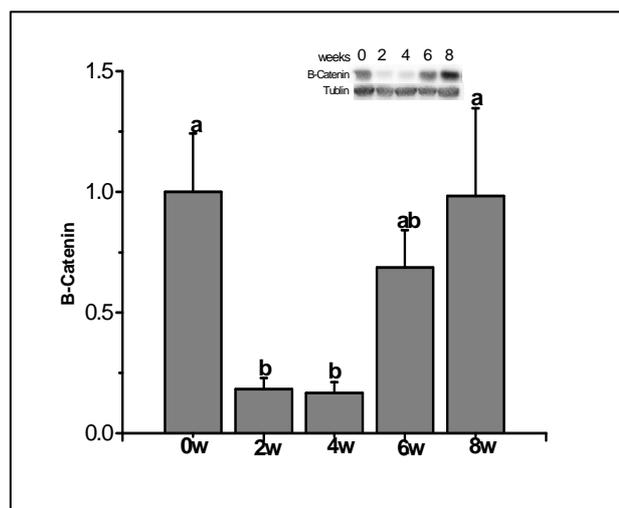


Figure 2: The level of β -catenin protein expression

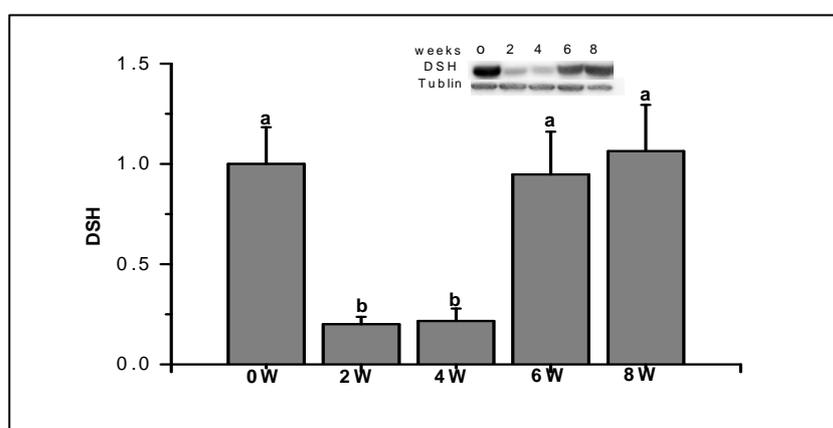


Figure 3: The level of DSH protein expression

In our study, we found that the Wnt-10b protein expression have close relationship with the hair follicle cycle of the Rex-rabbit. Meanwhile, the the β -catenin and DSH protein expression are similar to the Wnt-10b.

CONCLUSIONS

In conclusion, we investigated the Wnt-10b protein expression through the Western blot and Immunohistochemical as well the β -catenin and DSH protein expression. Our results suggest that Wnt-10b may play an important role during the hair follicle cycle of the Rex-rabbit through the Wnt/ β -catenin pathway.

ACKNOWLEDGEMENTS

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The Mechanisms of Hair Follicle Cycle Regulated by Wnt10b in Rex-rabbits from Birth to 2-month-old

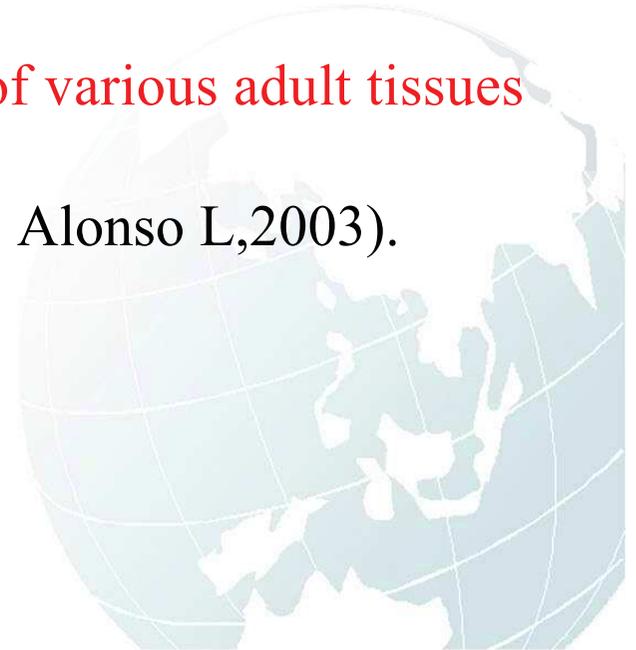
Repoter: Wu Zhenyu
Shandong Agriculture University
June 18, 2016





Background

- ✓ Wnt protein are secreted, lipid-modified glycoproteins and it can activate cell-surface receptor-mediated signal transduction pathways(Alonso L,2003);
- ✓ Wnt proteins are heavily involved in **morphogenesis during embryogenesis**, as well as in the **regeneration of various adult tissues** including **skin** and **hair follicles**(Bienz M,2000; Alonso L,2003).





Background

- ✓ Wnt signaling pathway is critical for the development and maturation of a variety of tissues, as well as stem cell maintenance, tissue development, and tumorigenesis.
- ✓ Wnt signaling pathway: **Wnt/ β -catenin** signaling pathway; Planar cell pathway; Wnt/ Ca^{2+} signaling pathway (Miller JR, 2002).





Background

- ✓ Mouse **Wnt10b** was initially isolated from lymphoid tissue and suggested to be involved in lymphoid development;
- ✓ Wnt10b play various roles in a wide range of biological actions, such as adipogenesis, bone formation, tumor development, and axis determination.
- ✓ Wnt10b was found to be expressed in **developing hair follicles**, with the earliest and most marked localization in placodes.

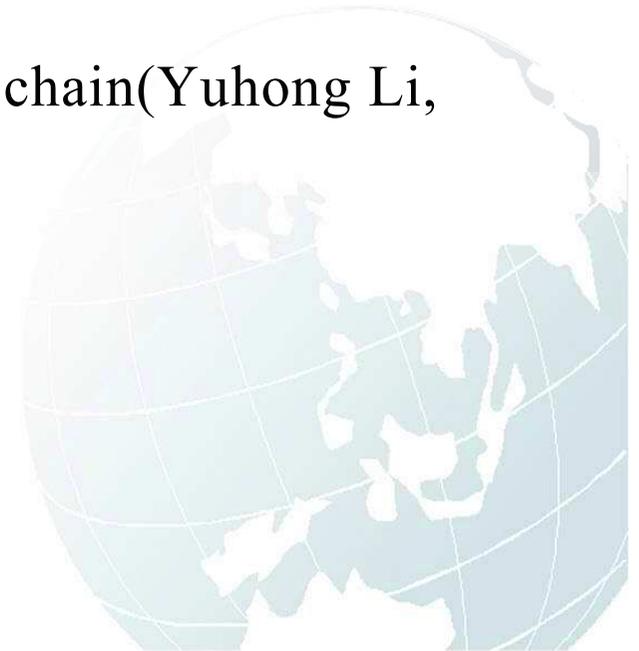




Background

✓ Research also found that Wnt10b can promote the differentiation of **skin epithelial** cells in vitro (Yukiteru Ouji, 2006).

✓ Wnt10b can induce mouse hair follicle regeneration through **Wnt10b-Frizzled-/ β -catenin-LEF/TCF** signal chain (Yuhong Li, 2011).





Experimental Procedures

Sampling skins from 0 weeks to 8 weeks old Rex-rabbit

Extracted total protein

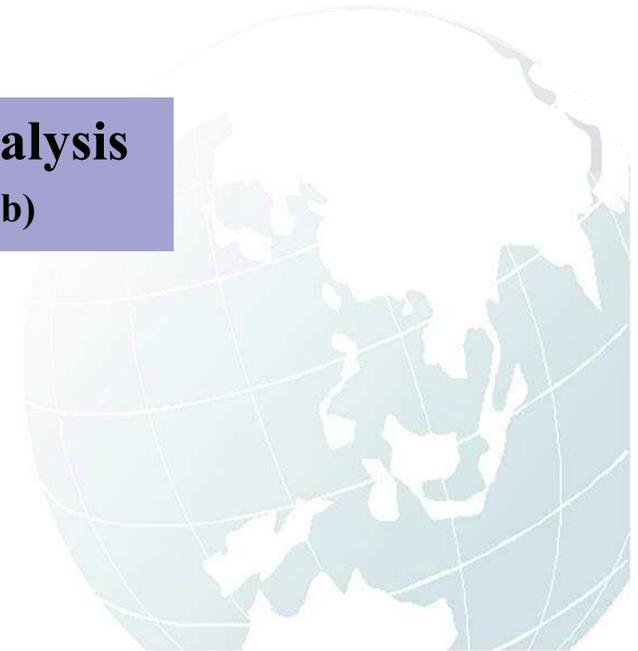
Paraffin embedding

WB analysis

(Wnt10b, β -catenin, DSH)

IHC analysis

(Wnt10b)





Results and Discussion

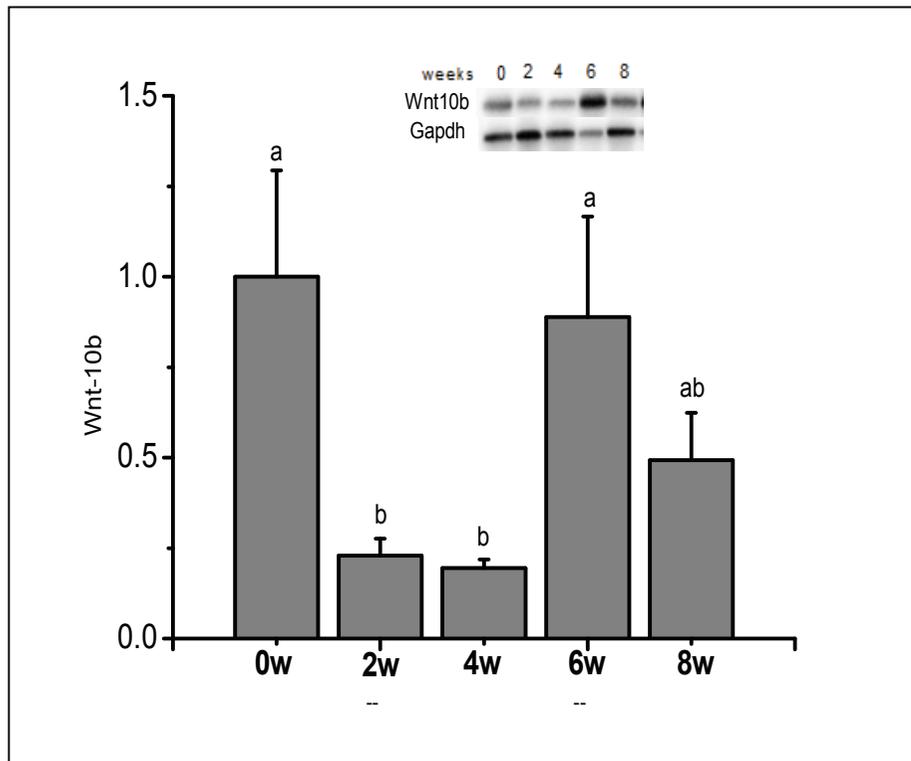


Fig1: The level of Wnt10b protein expression

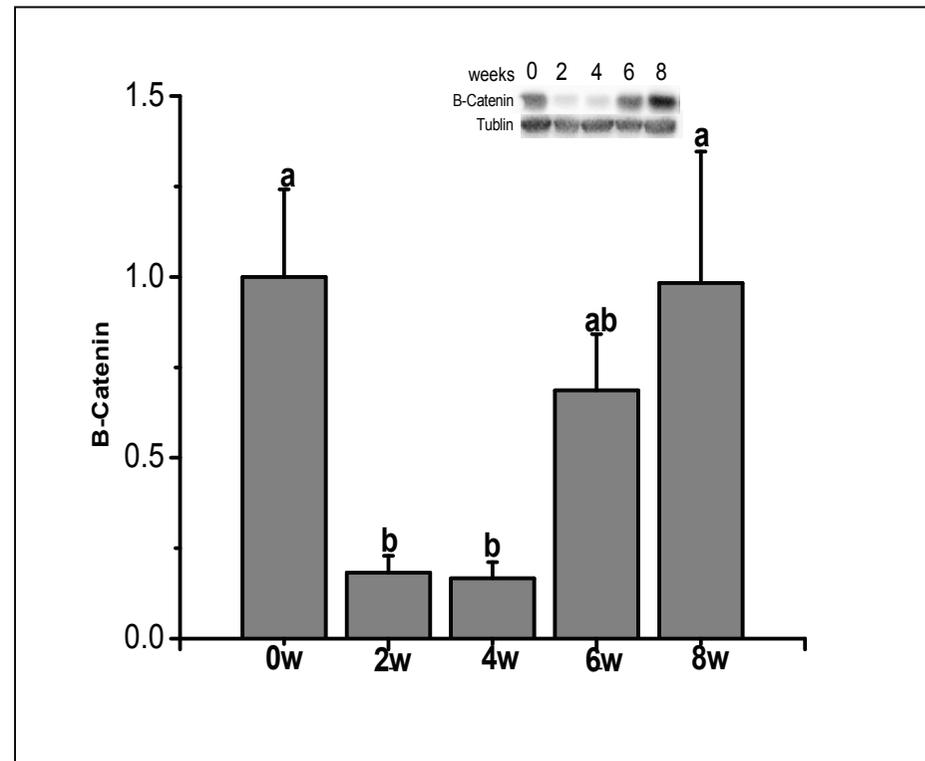


Fig2: The level of β -catenin protein expression



Results and Discussion

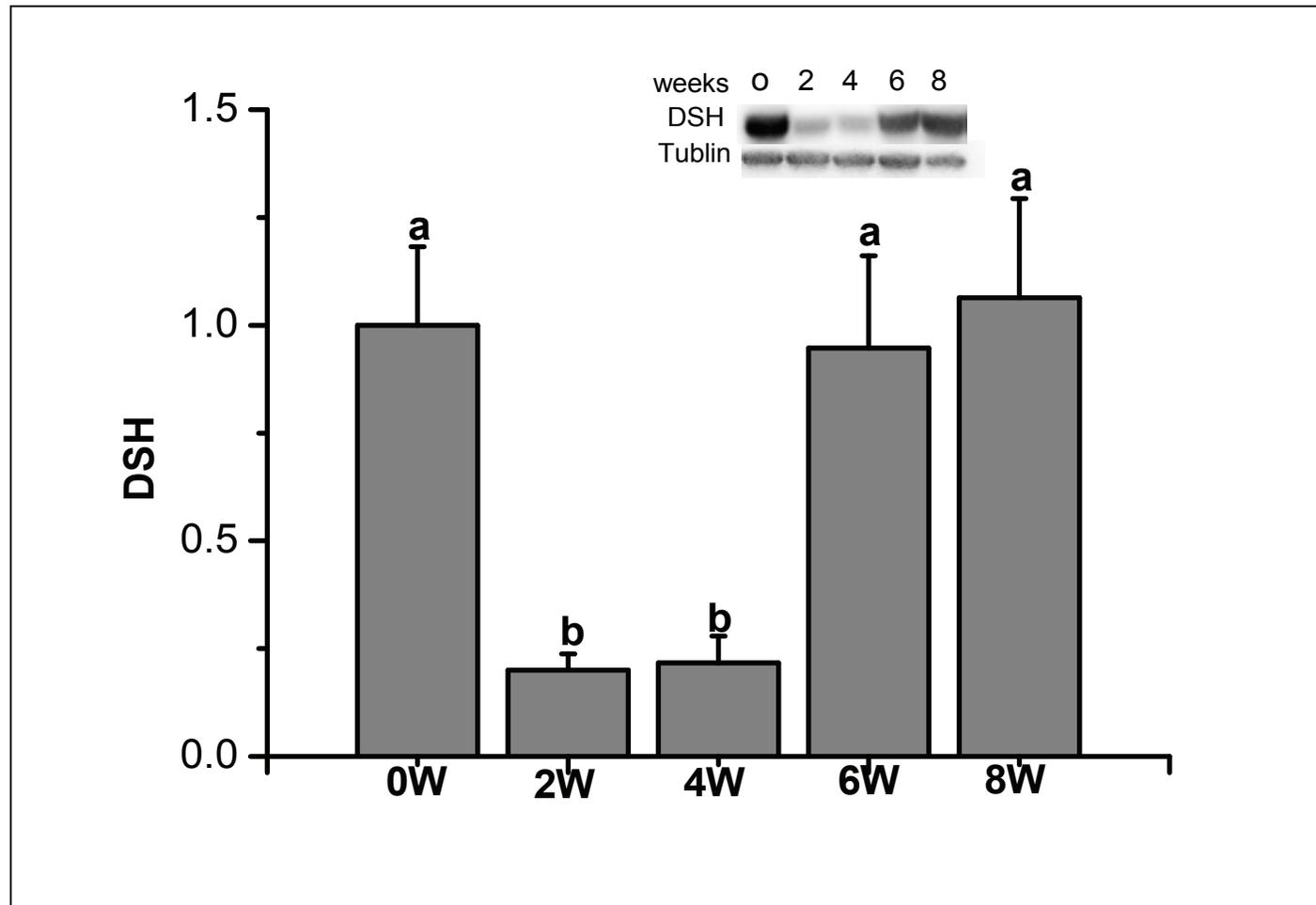


Fig3: The level of DSH protein expression



Results and Discussion

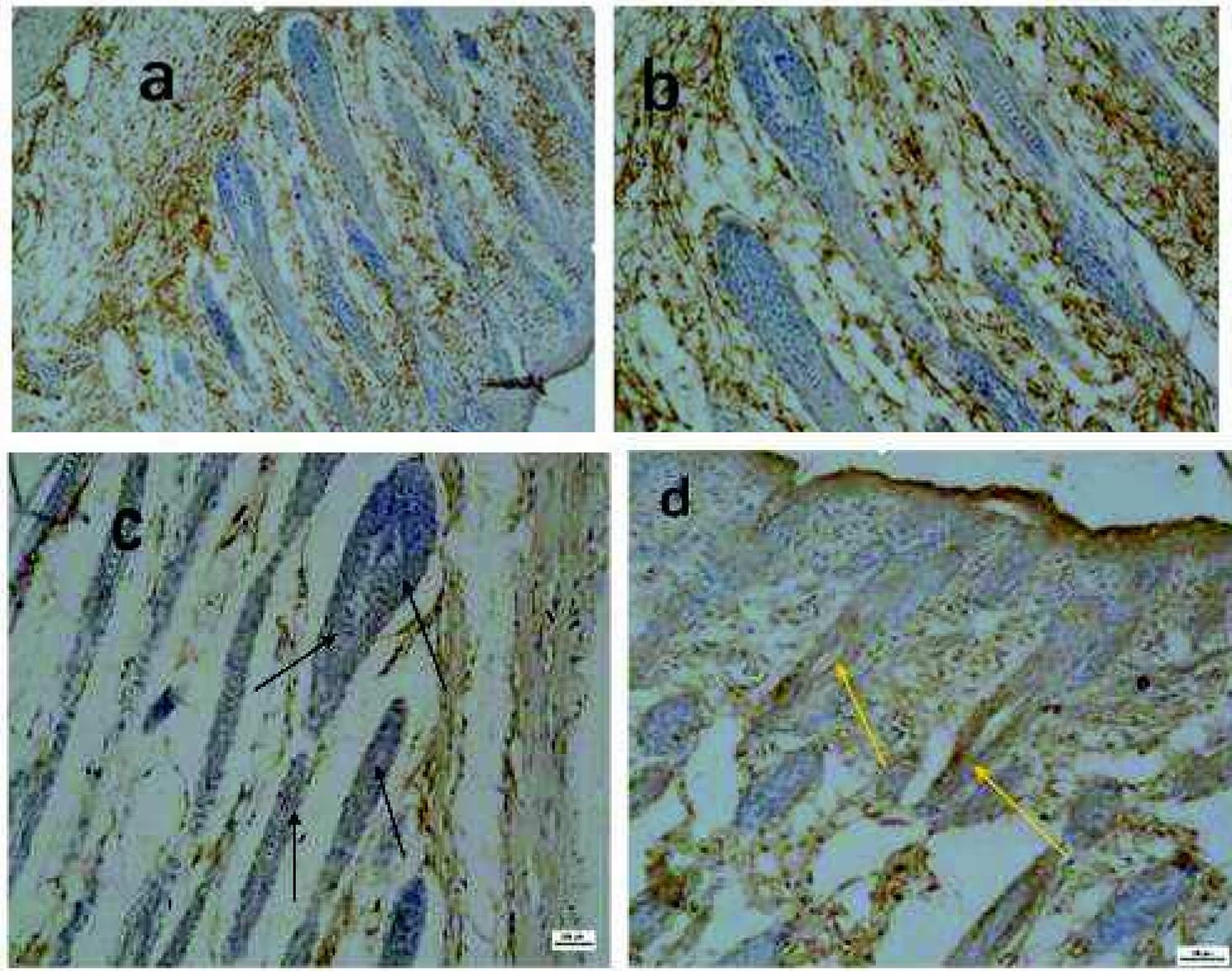


Fig4: Immunostaining of Wnt10b protein expression in the skins of Rex rabbit during 0~8 weeks of age (a,b X100; c,d X200)



Conclusions

(1) We found that Wnt10b protein express mainly in hair matrix, IRS and ORS of anagen hair follicle and express in IRS and ORS in catagen hair follicle. However, it is rare expressed in telogen hair follicle.

(2) Our result suggest that Wnt10b may play regulative role in the hair follicle through the Wnt/ β -catenin pathway





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